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CHONDRODYSTROPHY IN THE CHICK EMBRYO PRODUCED BY MANGANESE DEFICIENCY IN THE DIET OF THE HEN

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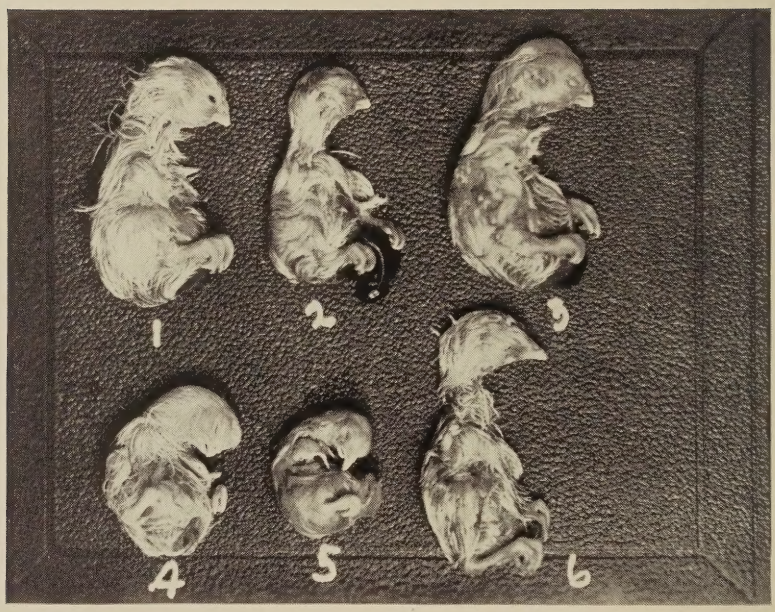

Figure 1. 1. Chondrodystrophic 21-day embryo. Note very short legs, "parrot beak," edema in the region above the atlas joint, globular contour of head and protruding abdomen.

2. Chondrodystrophic 20-day embryo. Note very short legs, "parrot beak" and globular contour of head

3. Chrondrodystrophic 21-day embryo. Note extreme edema involving the head and neck, very protruding abdomen, short legs and "parrot beak."

4. Chrondrodystrophic 21-day embryo. Note extreme shortening of mandibles and extreme edema of head and neck.

5. Chrondrodystrophic 19-day embryo. Note almost complete absence of down on the body small size, attachment of leg to the body from the tibial-metatarsal joint upward, and extremely short metatarsus.

6. Normal 21-day embryo.

Bulletin No. 371

Chondrodystrophy In The Chick Embryo Produced By Manganese Deficiency In The Diet Of The Hen

By MALCOLM LYONS and W. M. INSKO, JR.

Chondrodystrophy varies considerably in degree of expression as shown in Figures 1 and 2. The most common characteristics of chondrodystrophic embryos encountered in this study are (1) greatly shortened and thickened legs and shortened wings; (2) "parrot beak" resulting from a disproportionate shortening of the lower mandible and a downward curvature of the upper mandible; (3) globular contour of head apparently due to anterior bulging of the skull; (4) edema usually occurring just above the atlas joint of the neck and extending posteriorly for a variable distance, in some cases evident also in other parts of the body; (5) protruding abdomen apparently due to a relatively large amount of unassimilated yolk; (6) retarded down and body growth, particularly in the more severe cases (see Embryo No. 5, Figure 1).

Chondrodystrophy in the fowl seems to have been first reported by Landauer and Dunn (1926). Hutt and Greenwood (1929) found that the frequency of this abnormality from certain matings was as high as 34.78 percent of the eggs set in January but declined steadily to a complete absence in June. Munro (1932) found that the frequency of chondrodystrophy was definitely related to the season. The frequency of this condition decreased from 25.53 percent during the first part of March to 3.48 percent the latter part of May. Munro (1932) states " . . . the evidence is strong for believing the malformation to be due to genetic factors which depend upon unfavorable environmental conditions to become pheno-typically expressed."

Byerly, Titus and Ellis (1933) conclude that "Diets containing proteins from vegetable sources only increase the incidence of chondrodystrophy in the embryos of hens likely to produce such embryos." However, these authors did not find a seasonal distribu-

tion of this disorder as did Hutt and Greenwood (1929) and Munro (1932). Upp (1934) found the incidence of chondrodystrophy to be much higher in certain matings than in others, but no seasonal distribution of the disorder was noted. Byerly and co-workers (1935)

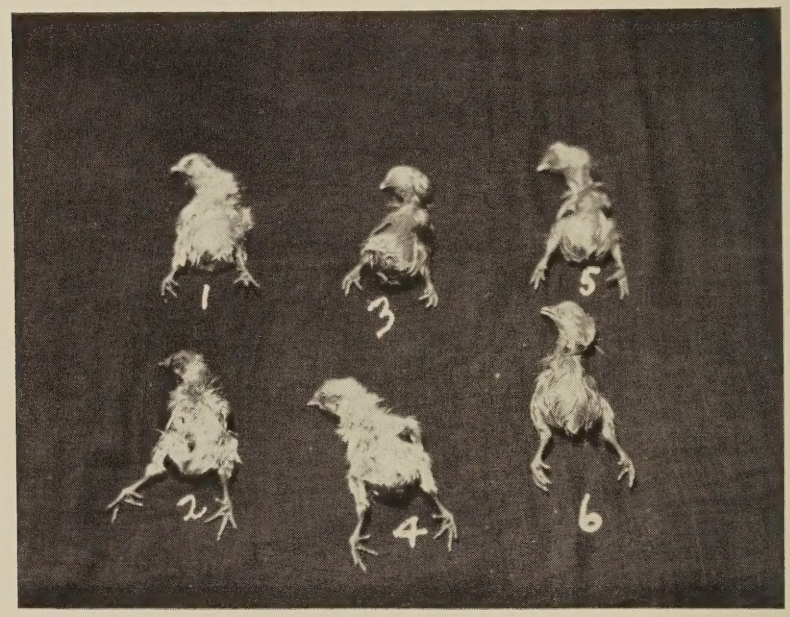

Figure 2. The effect of the injection of manganese directly into the egg, on embryonic development. Each consecutive pair (1-2, 3-4, and 5-6) is from eggs laid by the same hen within the same week. The eggs which produced embryos 1, 3, and 5 were not injected; those which produced 2, 4, and 6 were each injected with .03 mg of manganese. Numbers 1, 2, 4, and 6 are chicks, 3 and 5 are embryos that died on the 21st day of incubation.

report the occurrence of a type of chondrodystrophy distinctly different from the sporadic type referred to above, and have shown it to be definitely one of nutritional origin. These authors found that the disorder could be prevented by incorporating wheat germ and liver or wheat germ and whey in the ration of the parent stock. They also found that the nutritional chondrodystrophy could be prevented by allowing the parent stock direct sunlight and green range. Landauer (1936) gives an interesting histological description of the long bones in this disorder.

Patton (1937) reports that the glycine content of the muscles of

chondrodystrophic embryos (sporadic type) is much lower than that of the muscles of normal embryos.

The experiments herein reported were undertaken to determine the effect on embryonic growth and development, of feeding to laying hens a ration producing slipped tendon in chicks.

PROCEDURE

Sixteen Rhode Island Red pullets which had already started laying were selected from the Experiment Station flock and divided at random into two groups of eight pullets each. Each bird was placed in an individual compartment of an all-metal improvised hen battery, located in a room from which sunlight was excluded by covering the windows with tar paper. The room was artificially lighted twenty-four hours a day. An all-mash ration and tap water were given *ad libitum,* the water in glazed earthenware containers. The ration was of the following composition:

Ingredient	*Parts by weight*
Ground yellow corn	66.5
Dried skim-milk	15.0
Meat scrap	8.0
Steamed bone meal	5.0
Soybean oil	3.0
Sardine oil	2.0
Salt (NaCl)	0.5

This ration contained 1.4 percent phosphorus, 2.6 percent calcium and 5.5 parts per million (ppm) of manganese, and when fed to young chicks produces a high percentage of slipped tendon.

Lot 1 was fed the basal ration only, while Lot 2 received the basal supplemented by 40 parts per million each of manganese and zinc as the respective sulfates, and 100 parts per million of iron as ferrous ammonium sulfate. Manganese, zinc and iron were used in this connection because of the reported protective action of these elements in the prevention of slipped tendon (Wilgus and co-workers, 1936). Both lots were allowed oyster shell *ad libitum* but only a small amount of this material was consumed.

The eggs were gathered two or three times daily and pedigreed. After seventy-five days of this regime, each hen was stud-mated

every three or four days, usually with a White Leghorn male. Eleven males were used during the experiment. The males used at any given time were rotated between the pens each day. All eggs laid after the matings started were set at weekly intervals in one of three electric incubators and incubated under standard conditions of temperature and humidity for these machines. All eggs for each setting were incubated in the same machine and were hen-pedigree hatched. The eggs which failed to hatch were broken to determine fertility, the day of death of the embryo and its development.

RESULTS AND DISCUSSION

The hatchability and distribution of embryo mortality are shown in Table 1. The eggs produced by the hens fed the basal ration (Lot 1) gave a hatchability of only 5.2 percent as compared with 49.4 percent hatchability of the eggs from the hens receiving

Table 1. Hatchability and distribution of embryo mortality.

Lot No.	No. of fertile eggs	Treatment of eggs	Embryo mortality in percent of fertile eggs by weeks			Number of chicks	Percent hatchability
			1	2	3		
1	134	None	10.4	6.0	78.4	7	5.2
2	156	None	17.9	1.9	30.8	77	49.4
1*	25	None	20.0	8.0	68.0	1	4.0
1	21	Injected .03 mg Mn	19.0	0.0	42.9	8	38.1
1	6	Injected .03 mg Zn	0.0	0.0	100.0	0	0.0
2*	25	None	20.0	0.0	12.0	17	68.0
2	17	Injected .03 mg Mn	0.0	0.0	41.2	10	58.8
2	7	Injected .03 mg Zn	14.3	14.3	14.3	4	57.1

* These groups are tabulated separately from the bulk of the data of Lots 1 and 2 because they were the controls for the injection experiment.

Lot 1. Basal ration only. Lot 2. Basal ration plus 40 ppm each of Mn and Zn and 100 ppm of Fe.

the basal ration plus 40 ppm each of manganese and zinc and 100 ppm of iron. The seven chicks obtained from Lot 1 were all from the same hen and altho having short legs did not have slipped tendon. Seventy-eight percent (78.4%) of the embryos of the eggs from the hens in Lot 1 died during the last week of incubation, whereas only 30.8 percent of the embryos from Lot 2 died during the same week of incubation. Practically all embryos from Lot 1

that died during the last week of incubation did so on the 20th and 21st days. The embryos from the eggs of Lot 1 that died from the 10th thru the 21st day of incubation showed chondrodystrophy, while the embryos from the eggs in Pen 2 that died during this period showed normal skeletal development. These data are given in Table 2.

It seems clear from an examination of the data presented in Table 2 that this type of chondrodystrophy in the chick embryo is due to a mineral deficiency in the diet of the hen. Since Lot 2 was fed the basal ration supplemented with manganese, zinc and iron, it was not clear just which of these mineral elements was effective in the prevention of this disorder. Analyses of the eggs showed that those from the hens of Lot 1 contained much less manganese than those from the hens of Lot 2 which received the mineral supplement

Table 2. Incidence of short legs, "parrot beak," and edema.

Lot No.	Treatment of eggs	Dead embryos 10-21st day	Very short legs	Short legs	Parrot beak	Edema
			percent	percent	percent	percent
1	None	111	52.3	45.9	96.4	75.8
2	None	50	0.0	0.0	0.0	4.0
1*	None	19	57.9	36.8	100.0	57.9
1	Injected .03 mg Mn	9	0.0	0.0	0.0	22.2
1	Injected .03 mg Zn	6	83.3	16.7	100.0	83.3
2*	None	3	0.0	0.0	0.0	0.0
2	Injected .03 mg Mn	3	0.0	0.0	0.0	0.0
2	Injected .03 mg Zn	2	0.0	0.0	0.0	0.0

* These groups are tabulated separately from the bulk of the data of Lots 1 and 2 because they were the controls for the injection experiment.

Lot 1. Basal ration only. Lot 2. Basal ration plus 40 ppm each of Mn and Zn and 100 ppm of Fe.

(Table 4 to be discussed later). This together with the results of our studies on slipped tendon led us to suspect a manganese deficiency as the causative factor. Two methods are available for determining which element is responsible for the prevention of the abnormality. (1) By feeding the basal ration supplemented with the elements singly to groups of hens and determining the development of the embryos in the eggs thus produced. (2) By the direct injection of these elementts separately into eggs produced by hens fed the basal ration. The question could be answered most rapidly by the

latter method and the results obtained would perhaps offer more direct proof as to the deficiency. The latter method was employed with successful results.

The eggs laid in three consecutive weeks just prior to the termination of the feeding experiment were used in injection experiments. Those laid during the first two weeks of this three-week period were divided (by hens) at the end of each week into two groups as nearly equal as possible. One group was not injected (control)while .03 mg of manganese was injected directly into the albumen of each egg of the other. These eggs were placed in the incubator immediately following the injection. The eggs laid by each hen during the third week of this period were divided into three groups. One group of eggs was injected with .03 mg of zinc each, another with 03. mg of iron, while the third group was not injected. These mineral elements were injected as solutions containing 0.12 mg of the element per cc, manganese and zinc as sulfates and iron as ferrous ammonium sulfate. A small hole was drilled in the egg shell just over the air cell. The shell membranes were punctured with a small hypodermic needle and 0.25 cc (.03 mg of the element) of the particular solution injected directly into the albumen. A small circular piece of paper was sealed over the hole by the use of egg albumen. The eggs were incubated as previously described. Practically all the embryos from the eggs injected with iron died during the first week of incubation. The two embryos which lived beyond this state indicated that iron was not effective in the prevention of chon-

Table 3. Length of tibiae, metatarsi and humeri of chicks and 21-day embryos from eggs treated with manganese or zinc and from untreated eggs.

Lot No.	Treatment of eggs	No. chicks or 21-day embryos	Average length of tibiae	Average length of metatarsi	Average length of humeri
			mm	mm	mm
1	None	12	21.1	14.6	10.7
1	Injected .03 mg Mn	15	30.2	22.1	15.2
1	Injected .03 mg Zn	4	19.6	14.0	9.9
2	None	20	30.8	22.2	15.5
2	Injected .03 mg Mn	15	31.3	22.8	16.0
2	Injected .03 mg Zn	4	30.1	21.8	14.3

Lot 1. Basal ration only. Lot 2. Basal ration plus 40 ppm each of Mn and Zn and 100 ppm of Fe.

drodystrophy. Because of the small number these results are not tabulated.

The effects of other injections on hatchability and distribution of embryo mortality are shown in Table 1. The eggs from the hens of Lot 1, which were injected with .03 mgs of manganese per egg gave a hatchability of 38.1 percent as compared with 4 percent for the eggs not injected. Injections of .03 mgs of zinc did not improve the hatchability. The chicks which hatched from the eggs injected with manganese appeared normal in all respects.

Injections of zinc and manganese into the eggs produced by the hens (Lot 2) fed the mineral supplement did not improve hatchability or skeletal development.

All the embryos from the eggs of Lot 1 that were not injected showed chondrodystrophy whereas all the 10—21 day embryos and the chicks from eggs laid by the same hens during the same period but injected with .03 mgs of manganese per egg showed normal skeletal development. All the embryos from the eggs of this lot injected with zinc showed chondrodystrophy.

In order to obtain a more quantitative measurement of the effect of manganese on the skeletal development of the embryo, the tibiae, metatarsi and humeri of all chicks and 21-day embryos from the injection experiments were dissected out and the length carefully measured. These data are presented in Table 3.

The tibiae, metatarsi and humeri of the 21-day embryos and chicks from the eggs of Lot 1 which were injected with manganese were 51.4, 43.1 and 42.0 percent longer, respectively, than the same bones of those from the eggs which were not injected. The injection

Table 4. Manganese content of eggs as affected by the diet of the hen.

Sample	Sample collected days*	Manganese content in ppm of dry matter (100°) Lot 1	Lot 2
Egg yolk	27 – 28	0.90	1.28
Egg yolk	78 – 84	0.54	1.20
Whole eggs	85 – 91	0.56	0.96
Whole eggs	91 – 97	0.43	0.94
Whole eggs	98 – 104	0.54	0.84

* Number of days the hens had been on experiment when the samples were taken.

Lot 1. Basal ration only. Lot 2. Basal ration plus 40 ppm each of Mn and Zn and 100 ppm of Fe.

of zinc did not increase the length of these bones. The length of these bones of the embryos and chicks from the eggs of Lot 1 which were injected with manganese were essentially the same as those of the embryos and chicks from Lot 2, which was fed the mineral supplement. The disproportionate proximo distal reduction in the length of the long bones of the leg is interesting and is in agreement with the report of Landauer (1936) which states (in referring to chondrodystrophic embryos of this type) "In the legs the degree of reduction in the length of the long bones increases in the proximo distal direction." The effect of the injection of manganese into the egg, on skeletal development of the embryo is strikingly shown in Figure 2.

As stated earlier, manganese deficiency in the eggs of Lot 1 was suspected. Consequently composite samples of egg yolk and of

Table 5. Manganese content of the embroys, in parts per million of the material dried at 100° C.

Chondrodystrophic Lot 1			Normal Lot 2		
Number of embryos	Age days	Manganese ppm	Number of embryos	Age days	Manganese ppm
2	20	0.39	3	chicks	1.29
2	21				
4	20	0.38	3	21	1.49
4	21	0.62	3	21	0.98
4	21	0.54	4	21	1.19
1	20	0.52	3	21	0.95
4	21				
2	19	0.41			
4	21				
1	20	0.43			
4	21				
Average	—	0.47	Average	—	1.18

Lot 1. Basal ration only. Lot 2. Basal ration plus 40 ppm each of Mn and Zn and 100 ppm of Fe.

whole eggs (edible portion) from each of the two lots of hens collected at various times during the experiment were analyzed for manganese by the method of Skinner and Peterson (1930). Duplicate analyses were made. The results are presented in Table 4.

From an examination of these data it is readily apparent that the manganese content of the hens' diet very markedly affects the manganese content of the egg. This is one of the very few instances, wherein conclusive proof has been obtained of a deficiency of a mineral element in the diet of the hen being reflected by a restriction of the occurrence of the element in the edible portion (yolk and albumen) of the egg. The manganese content of eggs as reported in the literature varies greatly. Peterson and Skinner (1931) and McHargue (1925) reported, respectively, that egg yolk contained 2.3 and 1.5 ppm of manganese (dry matter basis). Bertrand and Medigreceanu (1920) and Peterson and Skinner (1931) reported, respectively, that whole eggs (edible portion) contained 2.52 and 1.1 ppm of manganese (dry matter). Part of this variation may be due to the different analytical methods employed. However, the hens producing these eggs were probably fed rations containing different amounts of manganese which would account for much of the variation.

Since the eggs which produced the chondrodystrophic embryos were shown to contain much less manganese than those in which embryonic development was normal, it seemed highly desirable to ascertain whether there was any difference in the manganese content of normal and chondrodystrophic embryos. Composite samples of chondrodystrophic (from Lot 1) and of normal embryos (from Lot 2) that died during the last three days of incubation were dried at 100° C after removal of all unassimilated yolk material from the body cavity, and analyzed for manganese by the method of Skinner and Peterson (1930). The findings are shown in Table 5. The manganese content of the chondrodystrophic embryos was consistently much smaller than that of the embryos having normal skeletal development. The thirty-two chondrodystrophic embryos which were analyzed contained an average of 2.4 micrograms* of manganese per embryo, whereas the thirteen embryos and three chicks showing normal skeletal development contained an average of 7.0 micrograms of manganese per embryo, thus affording further evidence that the malformation is caused by a deficiency of manganese in the egg.

* A microgram (γ) is one millionth of a gram (0.000001g).

GENERAL DISCUSSION

Several lines of evidence prove conclusively that the type of chondrodystrophy described in this paper is due to a deficiency of manganese in the egg. (1) The disorder was completely prevented by the addition of 40 ppm each of manganese and zinc and 100 ppm of iron to a ration which when fed to hens without the mineral supplement caused the production of eggs which gave chondrodystrophic embryos. Experiments now in progress show that the addition of 50 ppm of manganese but no zinc and iron to a ration containing approximately 5.5 ppm of manganese completely prevents chondrodystrophy in the embryo. (2) The hens from Lot 1, at the termination of the experiment, were transferred from the hen battery to a laying house on the Experiment Station farm. These hens were allowed direct sunlight and green range and were fed the regular Experiment Station laying ration, the mash of which is composed largely of wheat by-products and consequently contains a considerable amount of manganese. Forty fertile eggs produced by these hens between the fifteenth and thirty-third day, after removal from the batteries to these conditions, gave a hatchability of 87.5 percent, and all the chicks showed normal skeletal development. Thus it is shown that the hens soon cease the production of eggs producing chondrodystrophic embryos when proper alterations in the diet are made. (3) The complete prevention of chondrodystrophy by the injection of manganese directly into eggs which when not injected produced embryos showing this malformation, affords perhaps the most conclusive proof that this disorder is due to manganese deficiency in the egg. (4) Supporting evidence is afforded by the much smaller manganese content of the eggs from Lot 1 producing chondrodystrophy as compared with that of those from Lot 2 producing normal embryonic development, and by the much smaller manganese content of the chondrodystrophic embryos as compared with that of embryos having normal skeletal development.

The results of this investigation go far in the explanation of those obtained by Byerly and co-workers (1935). Before such an explanation becomes valid, it is essential to establish the identity of the disorder encountered in this investigation with that reported by these investigators.

The description given, and a comparison with the pictures shown, leave little doubt as to their identity. The peak of embryo mortality, short legs and wings, bulged head, parrot beak, straight tibiae and the disproportionate degree of reduction in the proximo distal direction in the length of the long bones of the legs described by Byerly and co-workers (1935) and by Landauer (1936) coincide with the characteristics of the chondrodystrophic embryos obtained in this study. The most characteristic differences between the sporadic and the nutritional type of chondrodystrophy aside from their histological differences, are that the former generally die during the second week of incubation, whereas, the latter show a peak of mortality during the last two or three days of incubation, and the sporadic type have bent tibiae while the tibiae of the nutritional type are not appreciably bent. In referring to these two types of chondrodystrophy Landauer (1936) states that "In many cases, . . . it would be difficult to distinguish these two types of malformed embryos on external appearance alone. Histologically these two types of micromelia are quite distinct."

Byerly and co-workers (1935) found that the nutritional type of chondrodystrophy could be prevented by allowing the parent stock direct sunlight and green range. It seems logical to assume that the hens on range consumed considerable green feed along with small amounts of soil and other materials containing considerable amounts of manganese, thus affording protection. The role of sunshine, if any, in the prevention of this disorder is not clear from the data available. The same authors found that meat meal and yeast were ineffective supplements for the prevention of the abnormality, whereas wheat germ was highly effective in this respect. These results seem easily explainable, since yeast and meat meal are relatively low in manganese as compared with wheat germ.

The same authors found that some of the short-leg embryos that hatch show unusual behavior in that there is a retraction of the head similar to that occurring in chicks with vitamin B deficiency. This condition was observed in one of the chicks (short legged) hatched from the eggs of the hens in Lot 1 (low manganese group). A large percentage of the short-legged chicks obtained in an investigation now in progress have a retraction of the head as described by Byerly

and co-workers (1935). This is additional evidence that the abnormality encountered by these investigators is identical with the one encountered in this investigation.

SUMMARY

The effect on embryonic development and hatchability of feeding to laying hens a ration producing slipped tendon in chicks, with and without a supplement of manganese, zinc and iron, is reported.

Eggs produced by hens fed the unsupplemented ration for 75 days gave a very low hatchability, with a peak of embryo mortality on the 20th and 21st day of incubation. The embryos that died after the tenth day of incubation were chondrodystrophic. Hens fed the same ration but supplemented with manganese, zinc and iron produced eggs giving good hatchability of normal chicks. The dead embryos from this lot showed normal skeletal development.

The hens fed the ration producing chondrodystrophic embryos, returned to the production of eggs supporting normal embryonic development within 15 days after they were transferred to farm conditions and allowed green range, direct sunlight and a ration containing considerable manganese.

The chondrodystrophic embryos were characterized by very short, thickened legs, short wings, "parrot beak," globular contour of head, protruding abdomen, and retarded down and body growth, particularly in the most severe cases. Very marked edema was noted in approximately 75 percent of these embryos.

The manganese content of the eggs producing chondrodystrophic embryos was much smaller than that of the eggs producing embryos of normal skeletal development.

Thirty-two chondrodystrophic embryos that died on the 19th, 20th and 21st day of incubation contained an average of 2.4 micrograms of manganese, whereas 13 embryos and 3 chicks showing normal skeletal development contained an average of 7.0 micrograms.

Chondrodystrophy was completely prevented by the injection of .03 mg of manganese directly into the albumen of eggs which when not injected produced embryos with this abnormality. The injection of zinc was ineffective.

The probable identity of this abnormality with that reported

by other investigators is discussed and a probable explanation of the results obtained by certain investigators is given.

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